

PURIFICATION OF A SUPERNATANT FACTOR THAT STIMULATES AMINO ACID TRANSFER FROM SOLUBLE RIBONUCLEIC ACID TO PROTEIN

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The reactions involved in the transfer of amino acids from soluble ribonucleic acid (sRNA) to protein are in need of clarification, and it would be of obvious advantage in studying this transfer to work with as clean a system as possible, both in regard to the particle preparation and in regard to any soluble components involved. A major effort in the Lipmann laboratory† has been directed toward identifying and purifying the factors in the 105,000 g supernatant fraction that stimulate amino acid transfer.¹

The initial observation by Hoagland *et al.*² of a stimulating effect of the pH-5 fraction on the transfer reaction in the rat liver microsomal system suggested that an enzyme or group of enzymes might be involved. Hülsmann and Lipmann,³ however, observed that sulfhydryl compounds, for example glutathione or cysteine, could partially replace the 105,000 g supernatant in stimulating the transfer of leucine from sRNA to the protein of rat liver microsomes. In many experiments glutathione and supernatant gave nearly the same degree of stimulation. It was therefore concluded that the effect of supernatant was due, in part, to its content of glutathione, glutathione reductase, and a TPNH-generating system. The clear recognition of a sulfhydryl requirement simplified the task of recognizing other, possibly enzymic, factors involved.

Although the addition of supernatant often had little effect beyond that of sulfhydryl compounds in our microsomal system, my associates and I still suspected that additional factors were involved. If so, however, the microsomes must have contained sufficient amounts to give a near-maximal rate of transfer. To probe further into this possibility deoxycholate-extracted microsomes were tried, for Kirsch⁴ at the Rockefeller Institute and Korner⁵ at Cambridge University, Cambridge, England had found that deoxycholate-treated liver microsomes retained the ability to incorporate amino acids. Our procedure was as follows: rat liver microsomes were prepared in Littlefield and Keller's⁶ medium A, using 24-hour fasted animals in order to deplete liver glycogen. The microsomes were well homogenized in 0.5 per cent deoxycholate and recentrifuged at 105,000 g for 2 hours. The pellets were rinsed several times with medium A and homogenized in the same medium. The specific activity of this preparation was about $1\frac{1}{2}$ times that of the original microsomes. This preparation withstood lyophilization and storage at -20° C. for as long as 3 weeks with little or no loss of activity.

In contrast to untreated microsomes, the deoxycholate-extracted particles showed an absolute requirement for the 105,000 g supernatant, even in the presence of glutathione, and the deoxycholate wash was found to contain a

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stimulatory factor. The almost complete dependence of this system on addition of supernatant is shown in FIGURE 1. This system thus could serve as a convenient assay system for supernatant factor, adjusting to values that fall

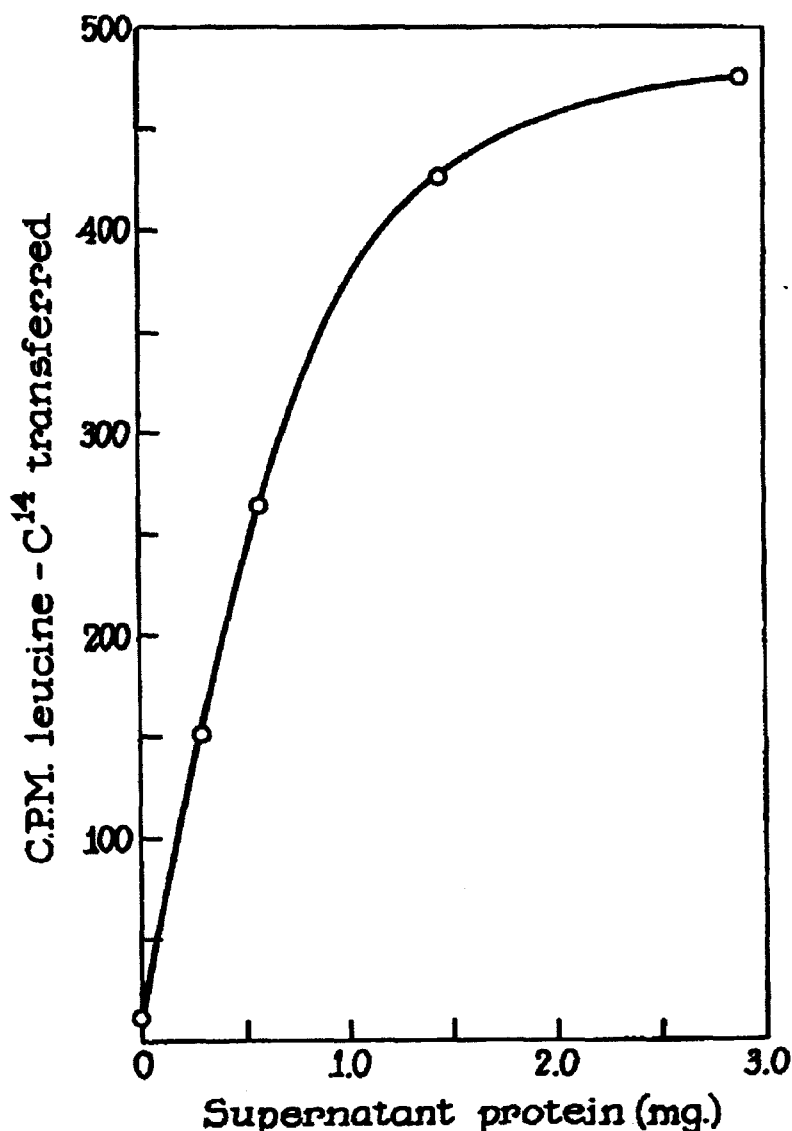


FIGURE 1. Dependence of the initial rate of leucine transfer on added 105,000 g supernatant. The incubation mixture contained the following in 1 ml.: deoxycholate particles (2.5 mg. protein); 0.29 mg. *Escherichia coli*-sRNA loaded with amino acids, including 1500 cpm leucine-C¹⁴ (10 μ C/ μ mole); 3 μ moles of ATP; 10 μ moles of phosphoenolpyruvate; 30 μ g. of PEP-kinase; 0.3 μ moles of GTP; 10 μ moles of glutathione; 100 μ moles of Tris HCl pH 7.0; 6 μ moles of MgCl₂; 50 μ moles of KCl; and supernatant as noted in the figure. After a 5 min. incubation at 35° C. 5 per cent trichloroacetic acid was added. The precipitate was extracted with 5 per cent TCA at 90° C. for 15 min., and washed with 5 per cent TCA and 3:1 alcohol-ether prior to plating for determination of radioactivity.

in the straight part of the curve. The supernatant factor is destroyed by heating at 60° C. for 5 min. and is nondialyzable. Ammonium sulfate and acetone fractionation resulted in a 30-fold purification with 30 per cent over-all recovery. The lability of the purified preparation at 0° C., however, is making further purification difficult.

FIGURE 2 shows the dependence of leucine transfer on added purified supernatant factor, and FIGURE 3 shows time curves for leucine transfer at two different concentrations of the purified fraction. In each case, 0.01 *M* glutathione was present. As noted in FIGURE 3, transfer is greatly reduced when glutathione is omitted. This glutathione effect, not apparent when whole

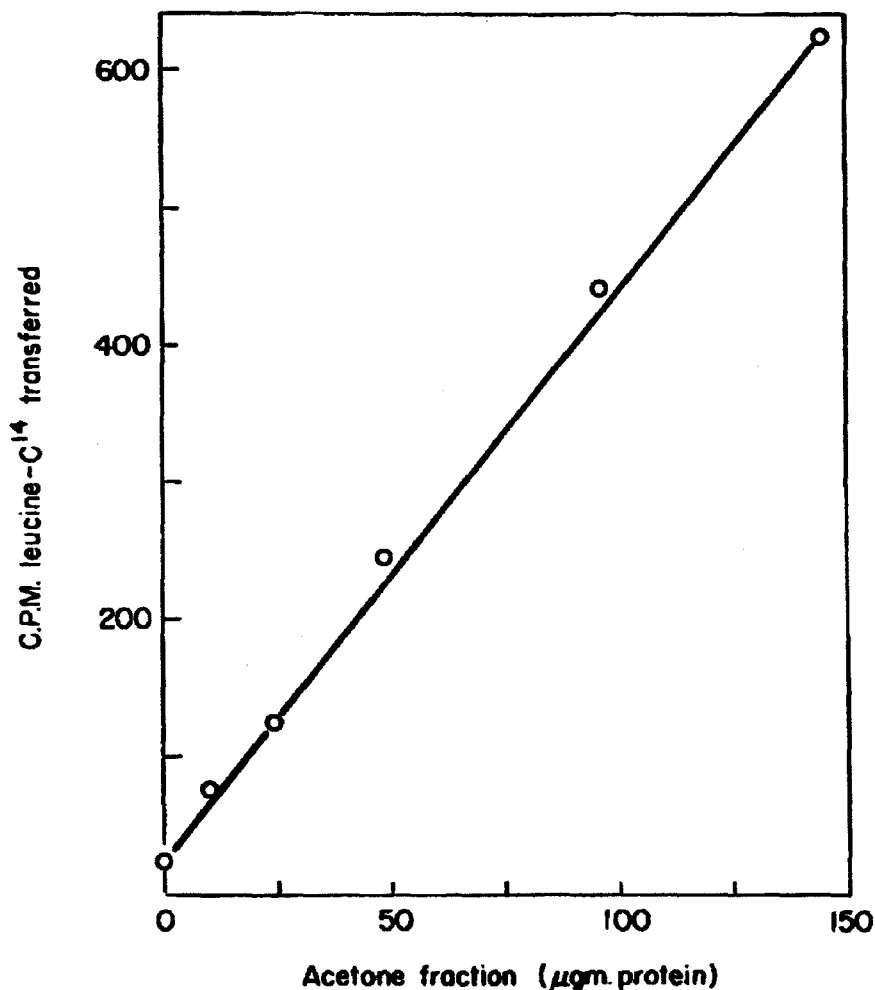


FIGURE 2. Dependence of the initial rate of leucine transfer on purified supernatant factor. The incubation mixture and conditions were the same as those given under FIGURE 1, except that 0.10 mg. of sRNA was present, and the specific activity of leucine was 25 $\mu\text{C}/\mu\text{mole}$. The purified preparation was the 26 to 32 per cent acetone fraction of the 39 to 50 per cent w./v. ammonium sulfate fraction. When this experiment was done, the purified preparation had lost one half its activity.

supernatant is used, is clearly brought out with the purified fraction. As with the untreated microsomes, cysteine is as effective as glutathione.

We conclude, therefore, that with this preparation a sulfhydryl compound and the partially purified, heat-labile, nondialyzable factor can replace the whole supernatant in stimulating leucine transfer from sRNA to the protein of microsomal particles. Although the results to date are consistent with an enzymatic action of the purified fraction, it is not yet certain that this factor is an enzyme.

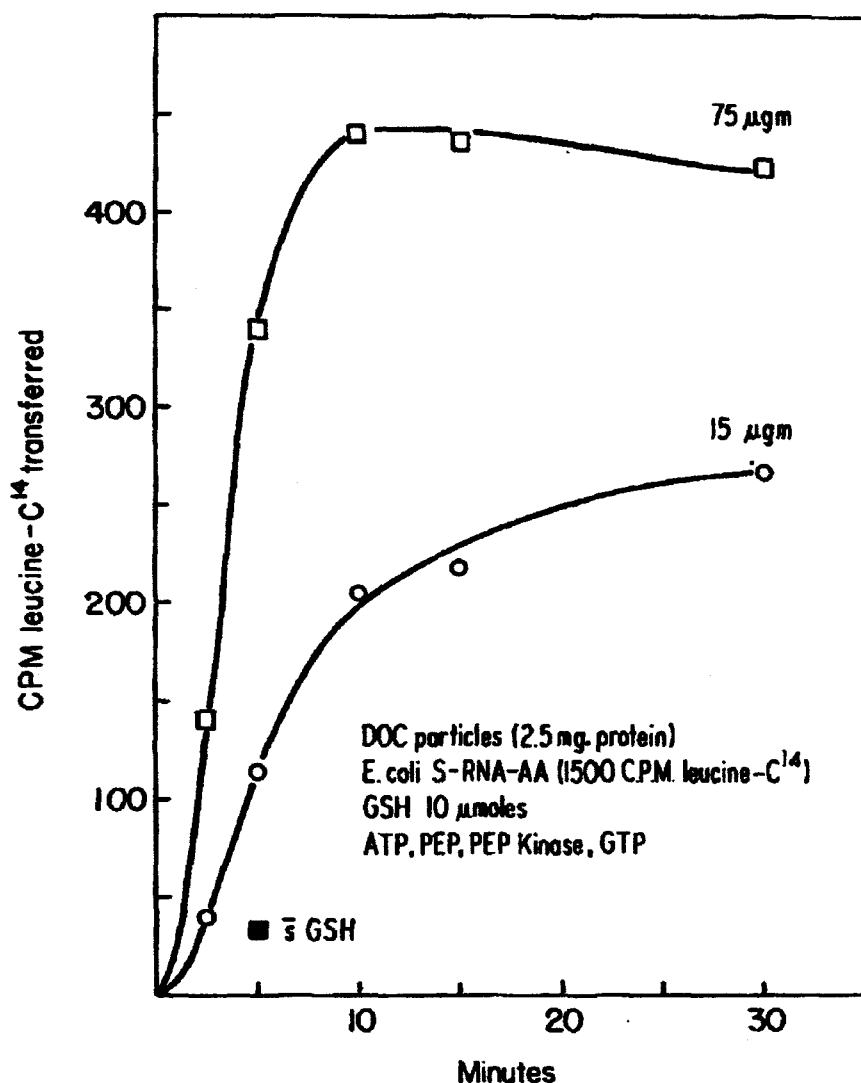


FIGURE 3. Time curves of leucine transfer with different amounts of the purified supernatant factor. The incubation mixture and conditions were the same as those given under FIGURE 1, except that 0.14 mg. of sRNA was present, and the specific activity of leucine was 25 $\mu\text{C}/\mu\text{mole}$. Lower curve, 15 μg . protein in the supernatant fraction; upper curve, 75 μg . The solid square indicates 5-min. value in the absence of glutathione, paired with the upper 5-min. point.

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